

Figure 1

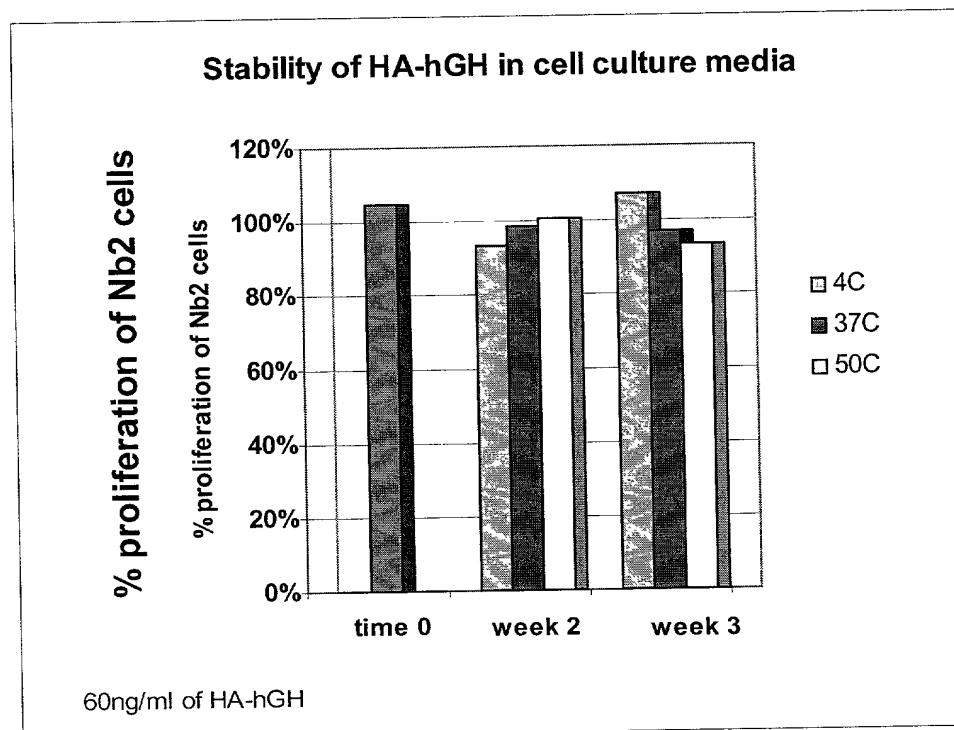


Figure 2

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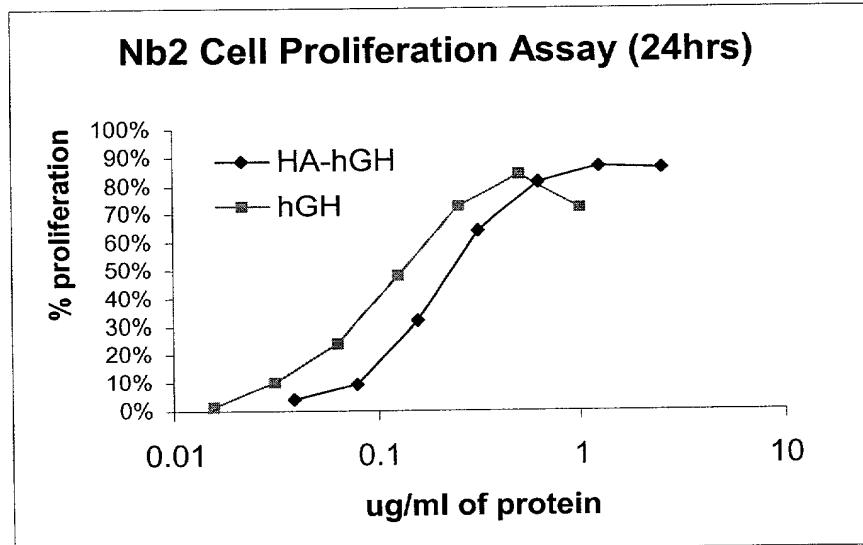


Figure 3A

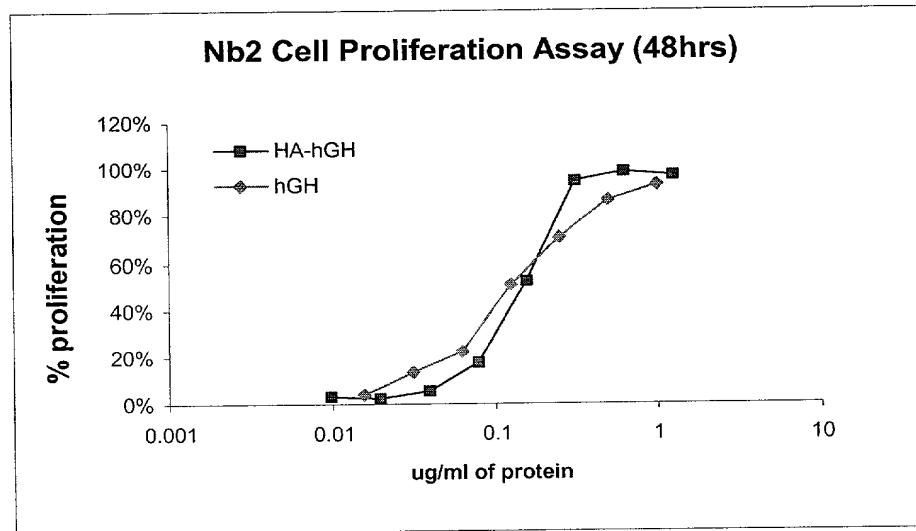
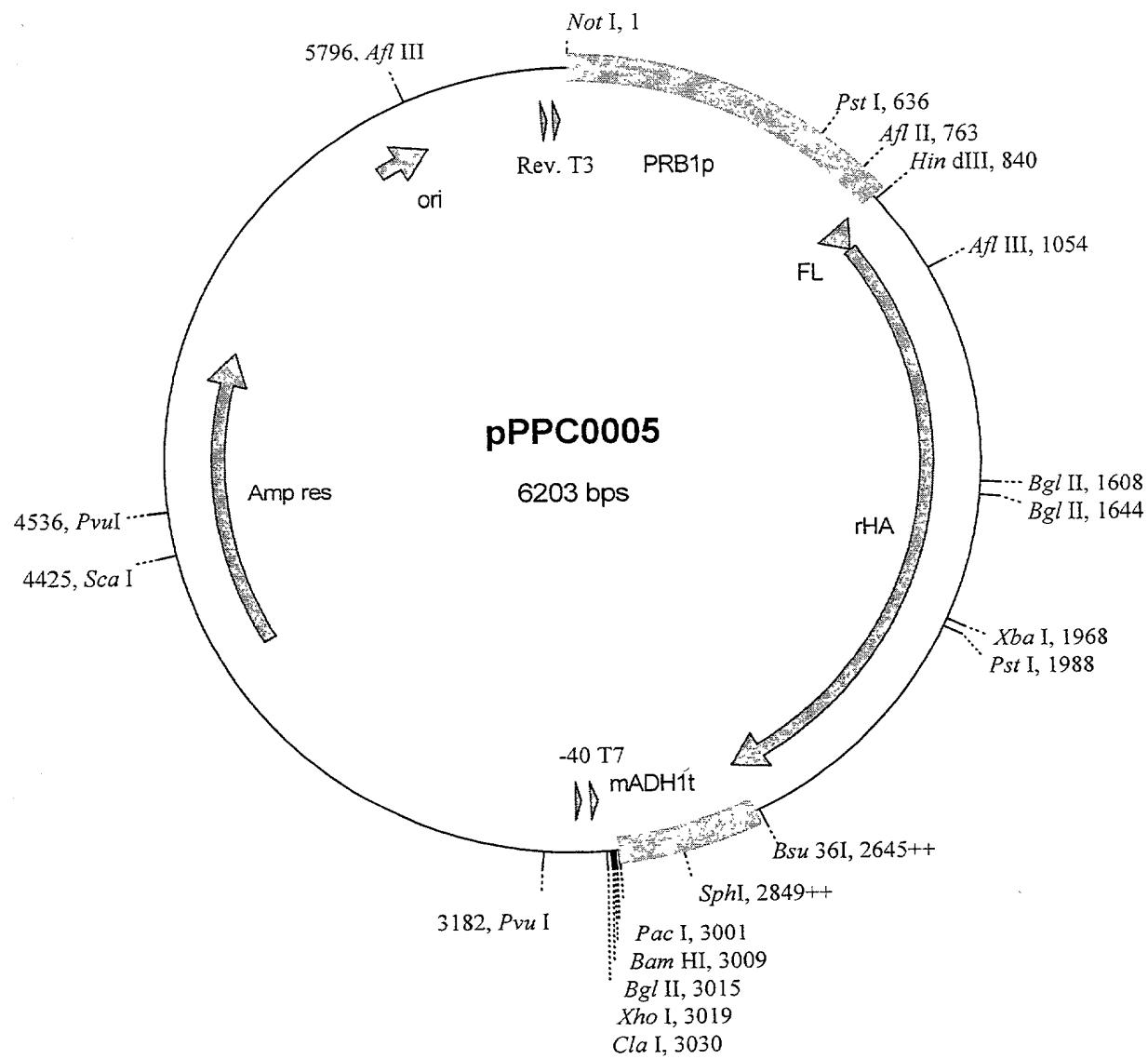


Figure 3B

**Figure 4**

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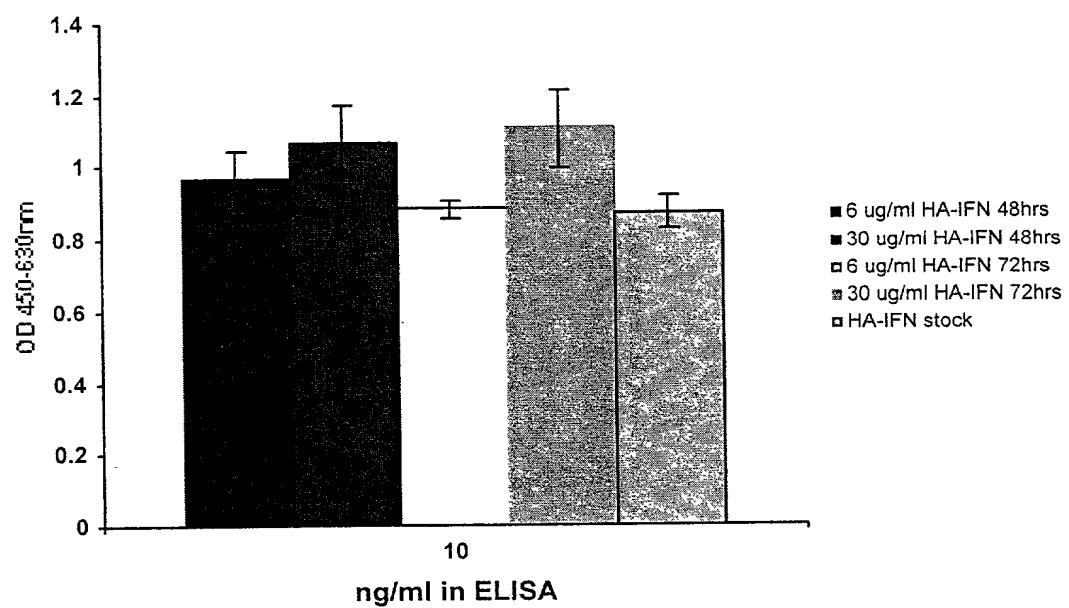
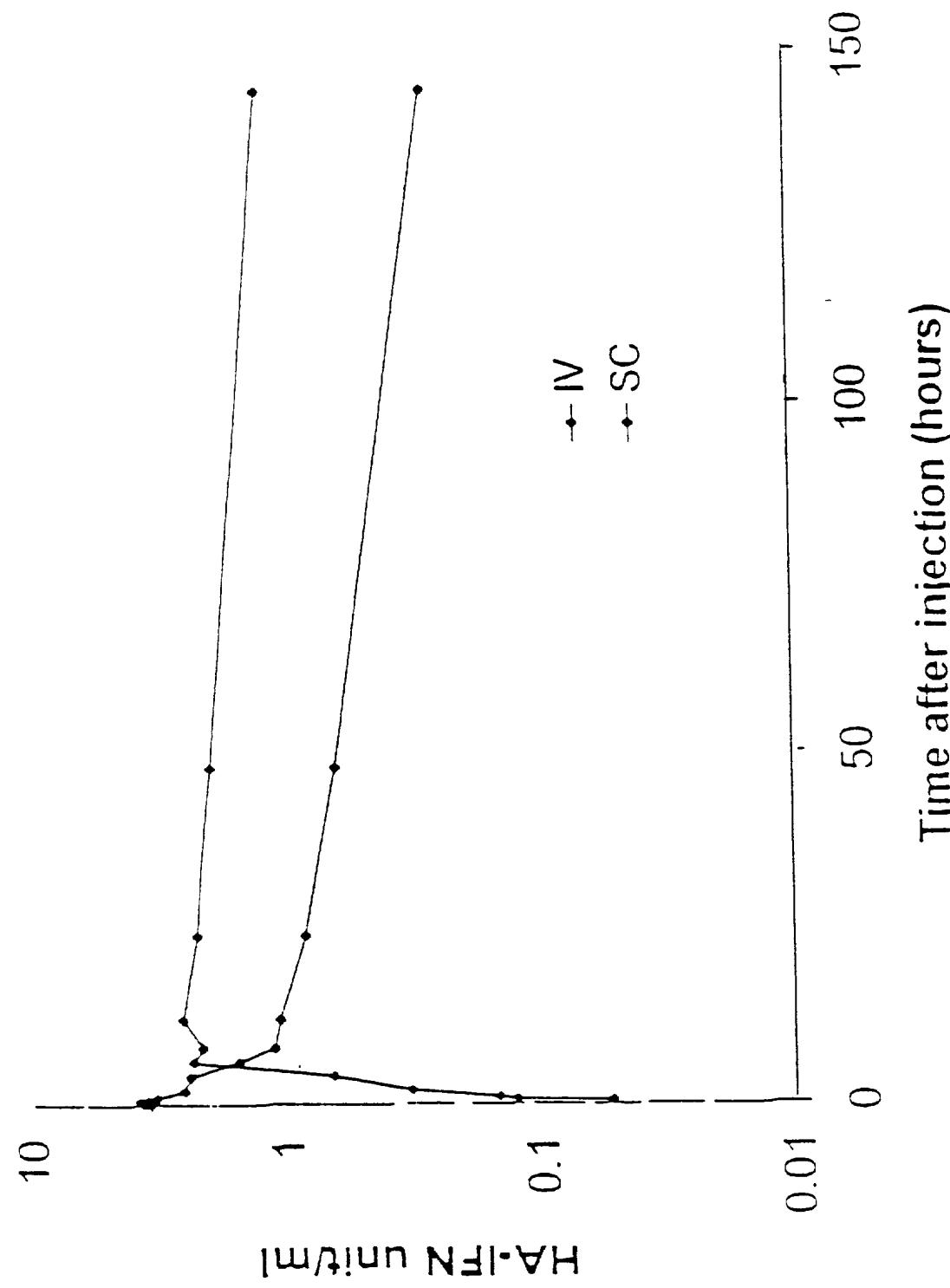


Figure 5

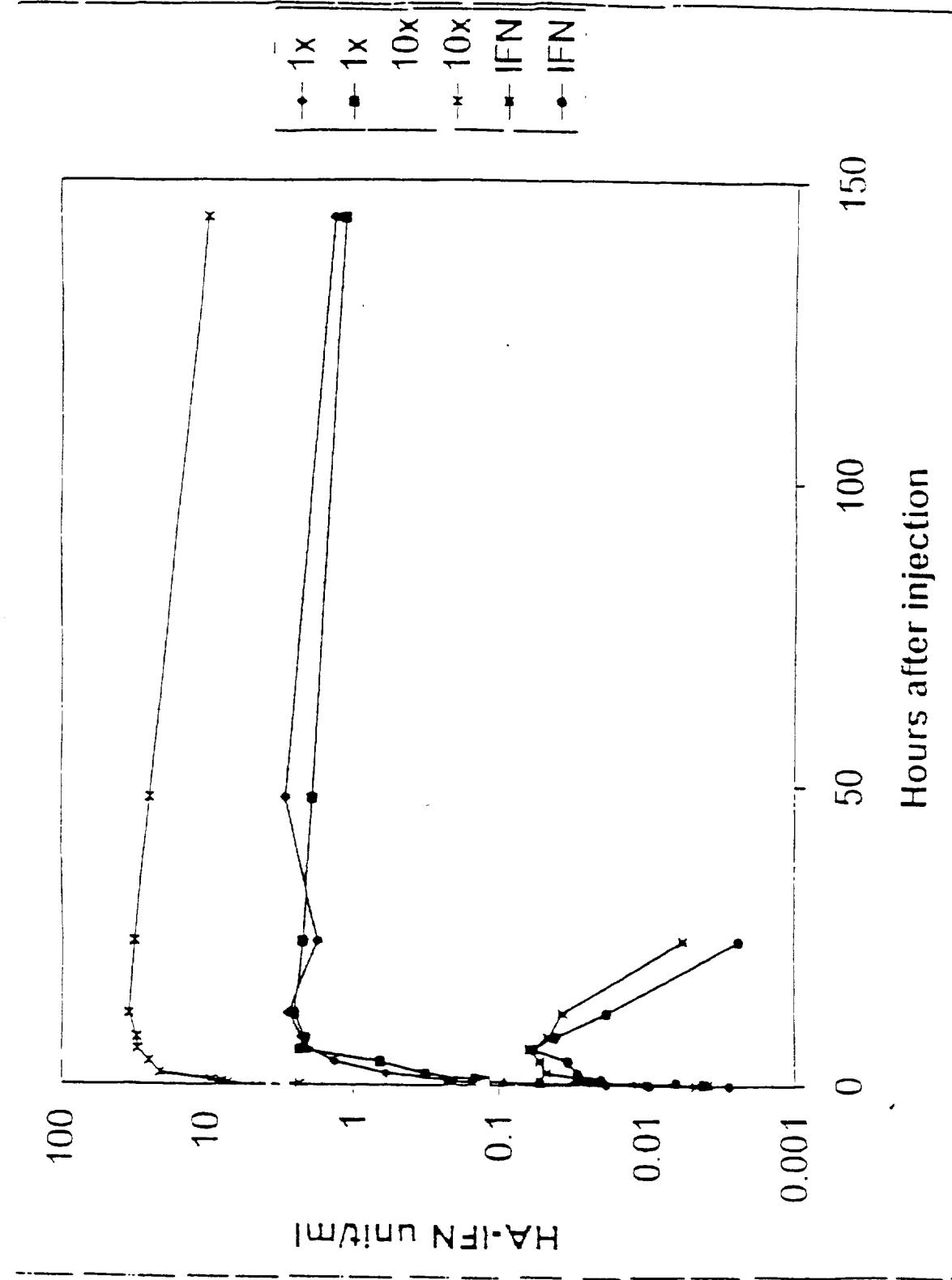
SCANNED, # 6

Figure 6



SCANNED, # 6

Figure 7



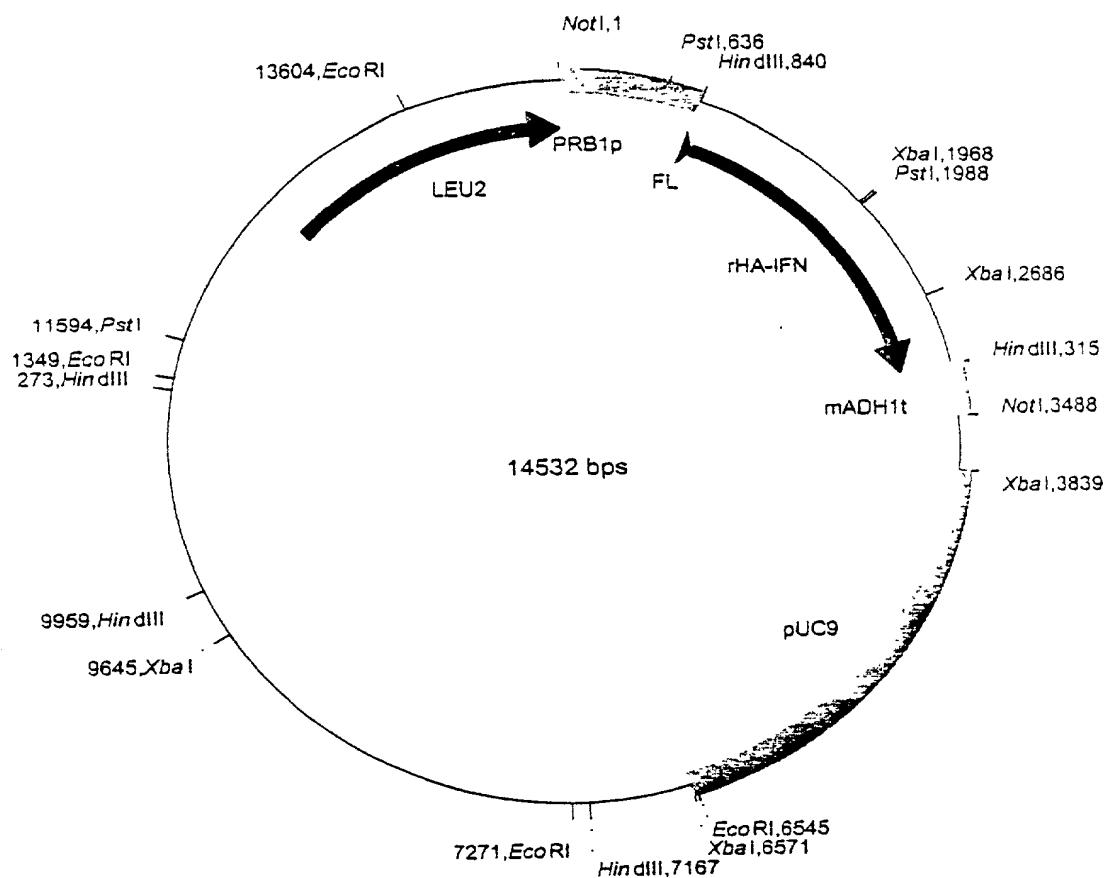


Figure 8. The HA-IFN α expression cassette in pSAC35. The expression cassette comprises
PRB1 promoter, from *S. cerevisiae*.
 Fusion leader, first 19 amino acids of the HA leader followed by the last 6 amino acids of the MFA-1 leader.
 HA-IFN α coding sequence with a double stop codon (TAATAA)
ADH1 terminator, from *S. cerevisiae*. Modified to remove all the coding sequence normally present in the *Hind III/Bam HI* fragment generally used.

Figure 8

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Localisation of ‘Loops’ based on the HA Crystal Structure which could be used for Mutation/Insertion

Loop		Loop	
I	Val154-Asn61	VII	Glu280-His288
II	Thr76-Asp89	VIII	Ala362-Glu368
III	Ala92-Glu100	IX	Lys439-Pro447
IV	Gln170-Ala176	X	Val462-Lys475
V	His247-Glu252	XI	Thr478-Pro486
VI	Glu266-Glu277	XII	Lys560-Thr566

Figure 9

Examples of Modifications to Loop IV**a. Randomisation of Loop IV.**

IV

151 APELLFFAKR YKAATTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC
 HHHHHHHHHHHH HHHHHHHHHH HHHHH HHHHHHHHHHHH HHHHHHHHHHHH

IV

151 APELLFFAKR YKAATTECCX XXXXXXCLLP KLDELRDEGK ASSAKQRLKC
 HHHHHHHHHHHH HHHHHHHHHH HHHHH HHHHHHHHHHHH HHHHHHHHHHHH

X represents the mutation of the natural amino acid to any other amino acid. One, more or all of the amino acids can be changed in this manner. This figure indicates all the residues have been changed.

b. Insertion (or replacement) of Randomised sequence into Loop IV.

$(X)_n$

↓

IV

151 APELLFFAKR YKAATTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC
 HHHHHHHHHHHH HHHHHHHHHH HHHHH HHHHHHHHHHHH HHHHHHHHHHHH

The insertion can be at any point on the loop and the length a length where n would typically be 6, 8, 12, 20 or 25.

Figure 10

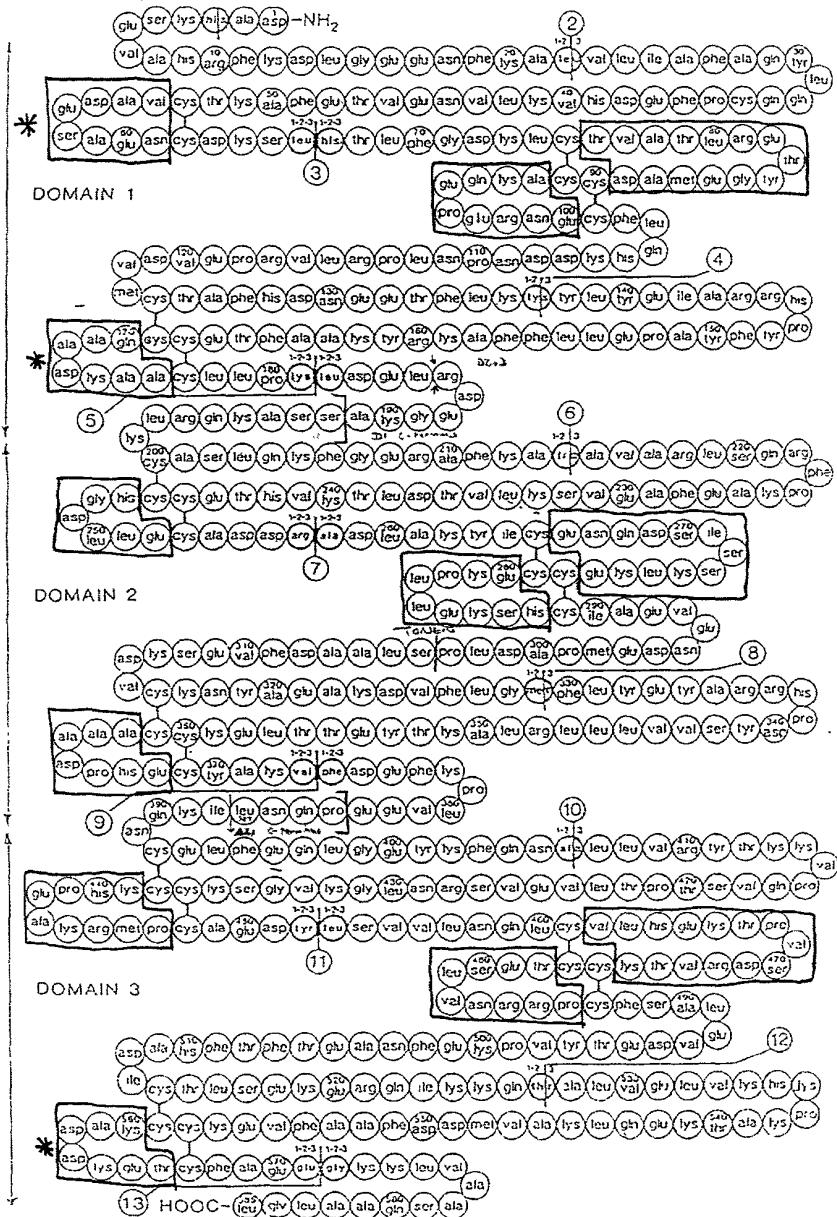
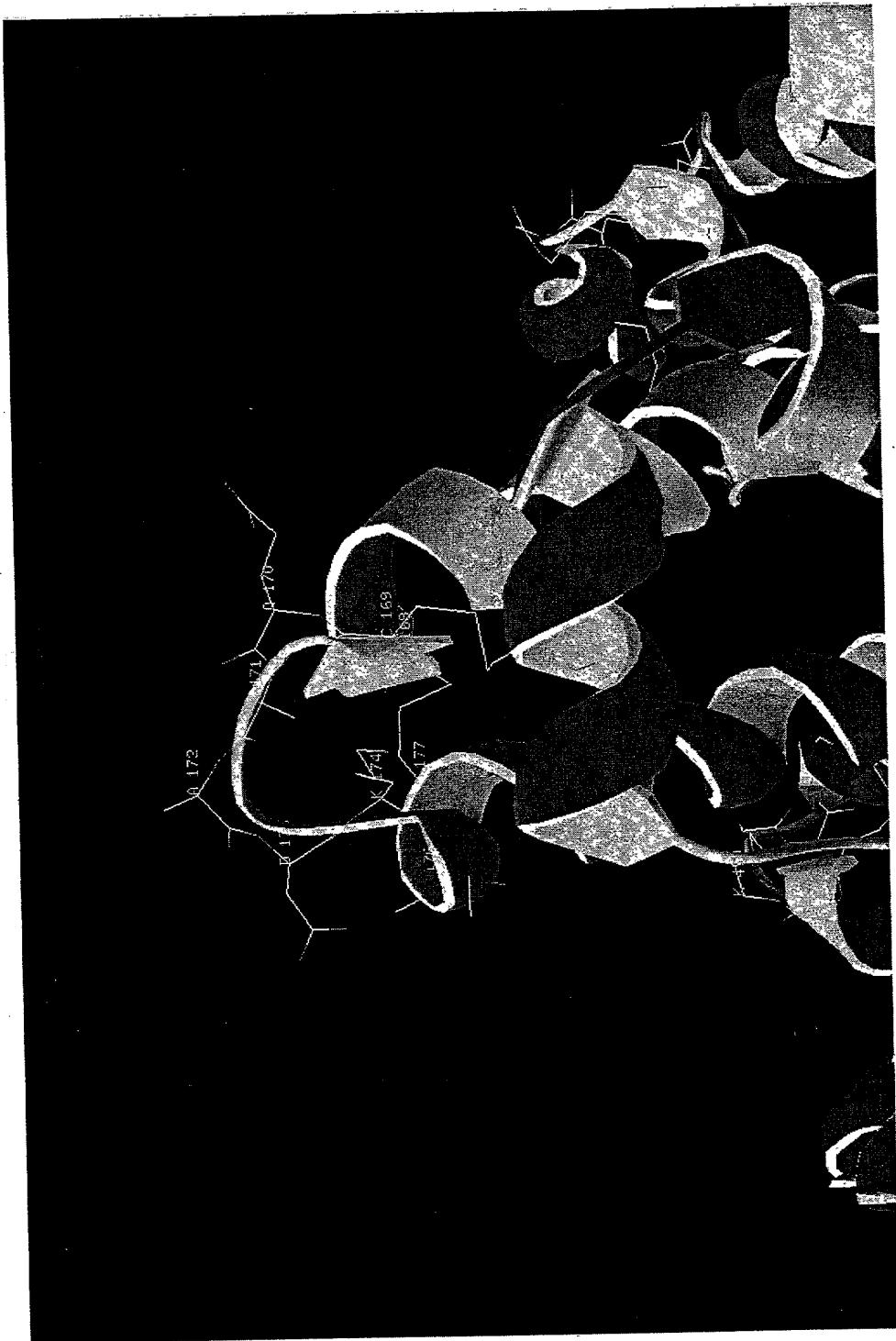
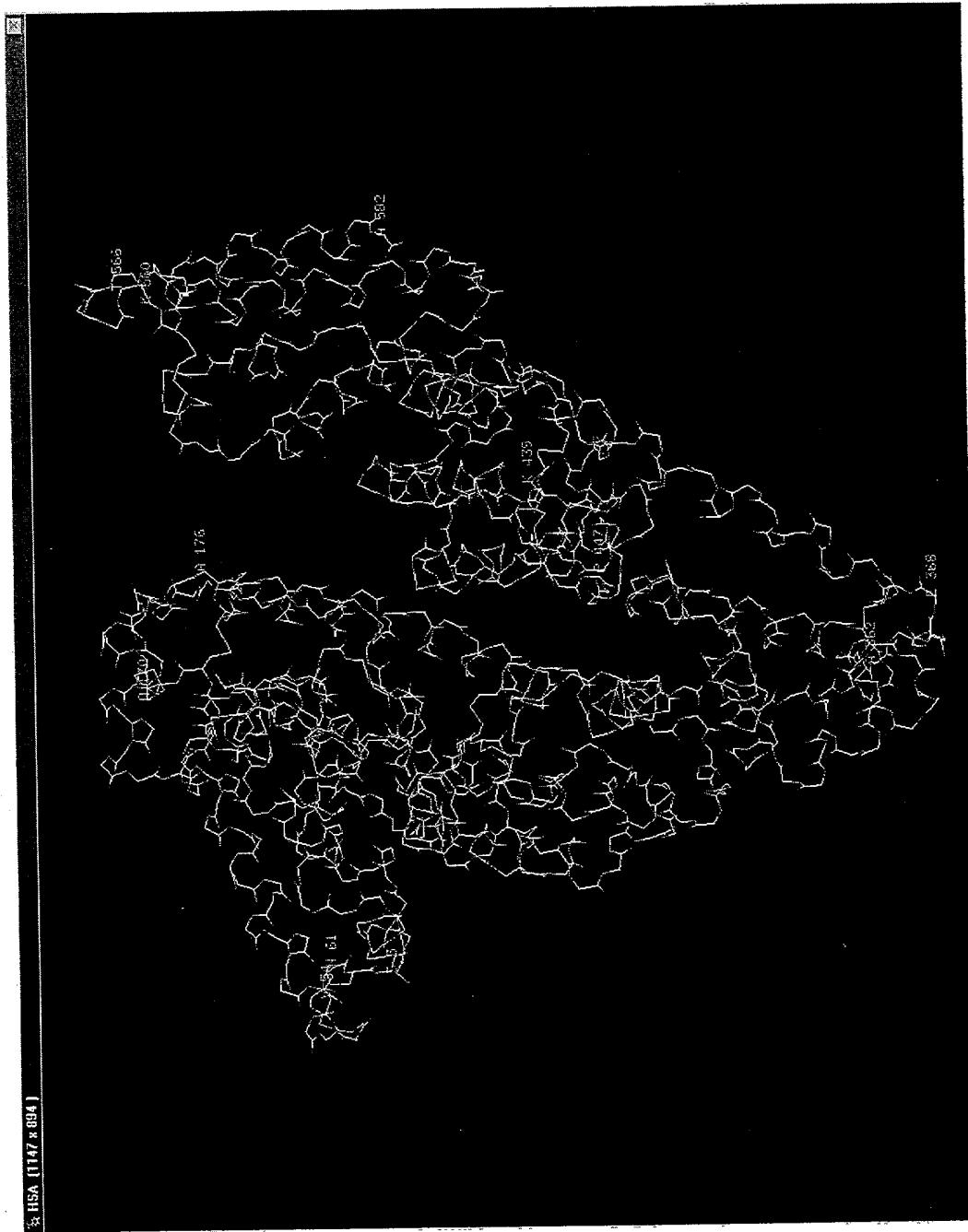


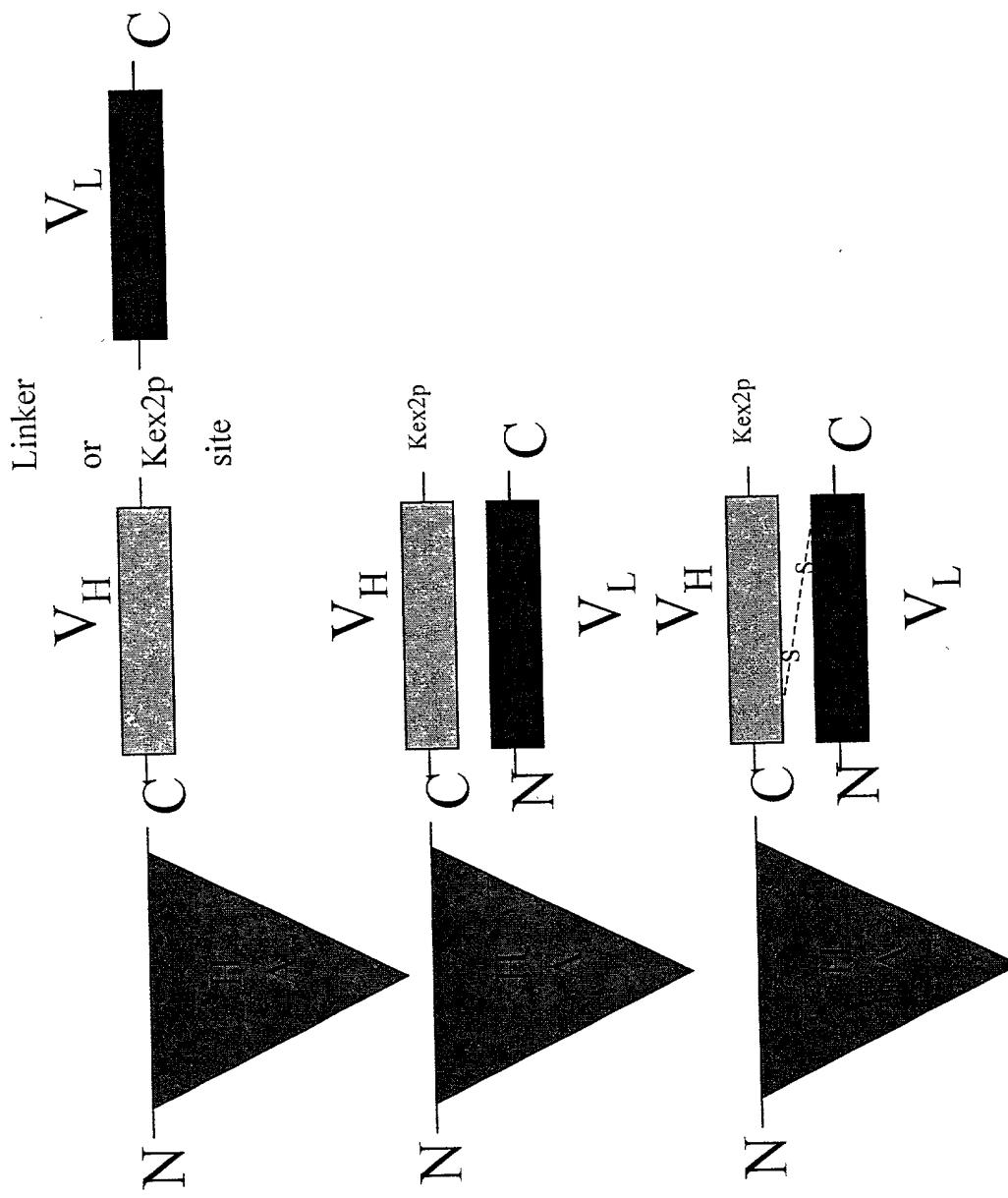
Figure 11



Disulfide bonds shown in yellow

Figure 12: Loop IV Gln170-Ala176





**Figure 14: Schematic Diagram of Possible ScFv Fusions
(Example is of a C-terminal fusion to HA)**

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1 GAT GCA CAC AAG AGT GAG GTT GCT CAT CGG TTT AAA GAT TTG GGA GAA GAA AAT TTC AAA 60
1 D A H K S E V A H R F K D L G E E N F K 20

61 GCC TTG GTG TTG ATT GCC TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAT CAT GTA 120
21 A L V L I A F A Q Y L Q Q C P F E D H V 40

121 AAA TTA GTG AAT GAA GTA ACT GAA TTT GCA AAA ACA TGT GTT GCT GAT GAG TCA GCT GAA 180
41 K L V N E V T E F A K T C V A D E S A E 60

181 AAT TGT GAC AAA TCA CTT CAT ACC CTT TTT GGA GAC AAA TTA TGC ACA GTT GCA ACT CTT 240
61 N C D K S L H T L F G D K L C T V A T L 80

241 CGT GAA ACC TAT GGT GAA ATG GCT GAC TGC TGT GCA AAA CAA GAA CCT GAG AGA AAT GAA 300
81 R E T Y G E M A D C C A K Q E P E R N E 100

301 TGC TTC TTG CAA CAC AAA GAT GAC AAC CCA AAC AAC CTC CCC CGA TTT GTG AGA CCA GAG GTT 360
101 C F L Q H K D N P N L P R L V R P E V 120

361 GAT GTG ATG TGC ACT GCT TTT CAT GAC AAT GAA GAG ACA TTT TTG AAA AAA TAC TTA TAT 420
121 D V M C T A F H D N E E T F L K Y L Y 140

421 GAA ATT GCC AGA AGA CAT CCT TAC TTT TAT GCC CCG GAA CTC CTT TTC TTT GCT AAA AGG 480
141 E I A R R P Y F A P E L L F A K R 160

Figure 15A

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481 TAT AAA GCT TTT ACA GAA TGT TGC CAA GCT GAT AAA GCT GCC TGC CTG TTG CCA 540
161 Y K A A F T E C C Q A A D K A A C L L P 180

541 AAG CTC GAT GAA CTT CGG GAT GAA GGG AAG GCT TCG TCT GCC AAA CAG AGA CTC AAA TGT 600
181 K L D E L R D E G K A S S A K Q R L K C 200

601 GCC AGT CTC CAA AAA TTT GGA GAA AGA GCT TTC AAA GCA TGG GCA GTG GCT CGC CTG AGC 660
201 A S L Q K F G E R A F K A W A V A R L S 220

661 CAG AGA TTT CCC AAA GCT GAG TTT GCA GAA GTT TCC AAG TTA GTG ACA GAT CTT ACC AAA 720
221 Q R F P K A E F A E V S K L V T D L T K 240

721 GTC CAC ACG GAA TGC TGC CAT GGA GAT CTG CTT GAA TGT GCT GAT GAC AGG GCG GAC CTT 780
241 V H T E C C H G D L L E C A D D R A D L 260

781 GCC AAG TAT ATC TGT GAA AAT CAG GAT TCG ATC TCC AGT AAA CTG AAG GAA TGC TGT GAA 840
261 A K Y I C E N Q D S I S K L K E C C E 280

841 AAA CCT CTG TTG GAA AAA TCC CAC TGC ATT GCC GAA GTG GAA AAT GAT GAG ATG CCT GCT 900
281 K P L I E K S H C I A E V E N D E M P A 300

901 GAC TTG CCT TCA TTA GCT GAT TTT GTT GAA AGT AAG GAT GTT TGC AAA AAC TAT GCT 960
301 D L P S L A D F V E S K D V C K N Y A 320

Figure 15B

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961 GAG GCA AAG GAT GTC TTC CTG GGC ATG TTT TTG TAT GAA TAT GCA AGA AGG CAT CCT GAT 1020
321 E A K D V F L G M F L Y E Y A R R H P D 340

1021 TAC TCT GTC GTC GTG CTG CTG CTG AGA CTT GCC AAG ACA TAT GAA ACC ACT CTA GAG AAG TGC 1080
341 Y S V V L L R L A K T Y E T T L E K P C 360

1081 TGT GCC GCT GCA GAT CCT CAT GAA TGC TAT GCC AAA GTG TTC GAT GAA TTT AAA CCT CTT 1140
361 C A A D P H E C Y A K V F D E F K P L 380

1141 GTG GAA GAG CCT CAG AAT TTA ATC AAA CAA AAC TGT GAG CTT TTT GAG CAG CTT GGA GAG 1200
381 V E E P Q N L I K Q N C E L F E Q L G E 400

1201 TAC AAA TTC CAG AAT GCG CTA TTA GTT CGT TAC ACC AAG AAA GTA CCC CAA GTG TCA ACT 1260
401 Y K F Q N A L L V R Y T K V P Q V S T 420

1261 CCT GAA GCA AAA AGA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GGC AGC AAA TGT TGT AAA CAT 1320
421 P T L V E V S R N L G K V G S K C C K H 440

1321 CCT GAA GCA AAA AGA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC AAC CAG TTA 1380
441 P E A K R M P C A E D Y L S V L N Q L 460

1381 TGT GTG TTG CAT GAG AAA ACG CCA GTA AGT GAC AGA GTC ACA AAA TGC TGC ACA GAG TCC 1440
461 C V L H E K T P V S D R V T K C C T E S 480

Figure 15C

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1441	T	TG	G	TG	AAC	AAC	AGG	CGA	CCA	TGC	TTT	TCA	GCT	CTG	GAA	GTC	GAT	GAA	ACA	TAC	GTT	CCC	AAA	1500
481	L	V	N	R	R	P	C	F	S	A	L	E	V	D	E	T	Y	V	P	K	500			
1501	GAG	TTT	AAT	GCT	GAA	ACA	TTC	ACC	TTC	CAT	GCA	GAT	ATA	TGC	ACA	CTT	TCT	GAG	AAG	GAG	1560			
501	E	F	N	A	E	T	F	T	F	H	A	D	I	C	T	L	S	E	K	E	520			
1561	AGA	CAA	ATC	AAG	AAA	CAN	ACT	GCA	CTT	GTT	GAG	CTT	GTG	AAA	CAC	AAG	CCC	AAG	GCA	ACA	1620			
521	R	Q	I	K	K	Q	T	A	L	V	E	L	V	K	H	K	P	K	A	T	540			
1621	AAA	GAG	CAA	CTG	AAA	GCT	GTT	ATG	GAT	GAT	TTC	GCA	GCT	TTT	GTA	GAG	AAG	TGC	TGC	AAG	1680			
541	K	E	Q	L	K	A	V	M	D	D	F	A	A	F	V	E	K	C	C	K	560			
1681	GCT	GAC	GAT	AAG	GAG	ACC	TGC	TTT	GCC	GAG	GAG	GGT	AAA	AAA	CTT	GTT	GCT	GCA	AGT	CAA	1740			
561	A	D	D	K	E	T	C	F	A	E	E	G	K	K	V	A	A	S	Q	580				
1741	GCT	GCC	TTA	GGC	TTA	TAA	CAT	CTA	CAT	TTA	AAA	GCA	TCT	CAG	1782									
581	A	A	L	G	L	*														585				

Figure 15D